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SHORT COMMUNICATION

Characterization of a porcine variable number tandem repeat sequence specific for the glucosephosphate isomerase locus

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Summary. A variable number of tandem repeat from a porcine glucosephosphate isomerase intron has been isolated and sequenced. The repeat has a unit size of 39 bp, is highly conserved and is present in at least 14 copies. Flanking sequences show a sequence periodicity of 53–54 bp and some sequence homology to the 39 bp repeat. A considerable part of the genomic DNA has been lost during subcloning and is considered to be deletion prone or refractory to propagation in *E. coli*. The tandem repeat is locus specific and detects at least six alleles in *Bam*HI digested porcine DNA. No homology to other tandem repeat sequences has been found.

Keywords: porcine, chromosome 6, glucosephosphate isomerase, variable number tandem repeat.

Many DNA sequences consisting of short (9–64 bp) motifs present in multiple copies and arrayed in tandem have been described, first in human DNA (Bell *et al.* 1982; Jeffreys *et al.* 1985a; Nakamura *et al.* 1987) and later in other mammalian and avian genomes (Jeffreys *et al.* 1985a; Vassart *et al.* 1986). These sequences have been termed 'minisatellites' (Jeffreys *et al.* 1985) or variable number tandem repeat sequences (VNTR) (Nakamura *et al.* 1987). Variation in the number of repeats gives rise to allelic variation which can be detected by Southern blotting using the repeat or flanking sequence as probes. The high degree of heterozygosity seen with tandem repeat DNA probes has made them extremely useful genetic markers. Some tandem repeats show homology to other loci, so one sequence can hybridize to several different loci, giving rise to 'genetic fingerprints' (Jeffreys *et al.* 1985a). One porcine 'minisatellite' sequence which detects 'fingerprints' at low stringency has been characterized (Coppieters *et al.* 1990). This work describes a porcine 'minisatellite' which is locus specific.

A 6-kb *Pst*I fragment was isolated from lambda GPI8 (Davies *et al.* 1988) and subcloned in pSKM13 (Stratagene) to give pGPIP. The plasmid pGPIP was digested

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to completion with *Not*I or *Xho*I and the ends protected by 'Klenow' filling with thio deoxy nucleotides. The *Not*I and *Xho*I cut plasmids were digested with *Sma*I and *Eco*RI, respectively, prior to the generation of ordered deletions using Exonuclease III and S1 nuclease, as described by the supplier of the kit used (Pharmacia, Uppsala, Sweden). The deletion plasmids were recircularized by ligation, transformed into *E. coli* XL1-Blue (Strategene, La Jolla, CA, USA) and selected on LB ampicillin plates. Mini-preps were prepared by standard methods (Birnboim & Doly 1973). The extent of the deletion was determined by running *Pvu*II digested plasmid on 1% agarose gels with *Hin*dIII digested lambda size markers.

Manual sequencing using plasmid DNA was carried out using T7 polymerase according to the instructions of the supplier of the kit used (Pharmacia). Automated DNA sequencing was as described by Kristensen *et al.* (1988). The products were analysed and the sequence was derived using an instrument constructed at EMBL, Heidelberg, Germany (Ansorge *et al.* 1986, 1987). DNA sequences were aligned and analysed using DNASIS software (Hitashi America Ltd, San Bruno, CA, USA).

Genomic DNA was isolated from EDTA-treated blood from Norwegian Landrace pigs by standard methods (Miller *et al.* 1988). DNA was digested with *Bam*HI, separated on 0·7% agarose gels and transferred to Hybond N plus (Amersham, Amersham, Bucks, UK) by alkaline transfer (0·4M NaOH). Prehybridization and hybridization were carried out at 65°C according to the manufacturer's recommendations. Filters were washed three times in 2XSSC, 0·1% SDS at 55°C or 65°C, low and high stringency, respectively, with a final wash in 0·1XSSC, 0·1% SDS at 65°C for high stringency washing. Filters were exposed to X-Omat film (Kodak) for 12–36h with intensifying screens.

The tandem repeat hybridization probe was prepared by *Pvu*II digestion of a deletion clone pPD05. Separation of the relevant DNA band was done by preparative low melting point agarose gel electrophoresis and purified using 'Geneclean' (BIO 101, La Jolla, California). Radioactive labelling was carried out by the random priming method (Feinberg & Vogelstein 1983).

The tandem repeat (Fig. 1) consists of a 39 bp unit present in near perfect repeats. Figure 1 shows 8 units of the repeat. More have in fact been sequenced but these are not detected as individual units by the sequence alignment software, which treats perfect repeats as overlapping sequences. This is further supported by the autoradiographs from the manual sequencing which show that the repeat pattern, easily recognizable by the 3′ CCCTGGG 5′ motif, is present in at least 14 copies in one clone (data not shown). Lastly the sizes of the cloned inserts containing the 39-bp repeat were estimated, by restriction digest analysis (results not shown), to be in the region of 1kb. Flanking sequences consisted of divergent repeats with a 53/54-bp periodicity. All subclone sequences were positioned according to insert size and sequence homology to overlapping clones. Several clones could not be linked to the sequence. This was not in accordance with the insert size data which indicated that link-up should have been detected. These results would imply some form of rearrangements or deletions during subcloning. Only sequences for which a number of overlapping clones are available and for which insert size correlates are presented in Fig. 1.

```
5′ TAGAGCTAGC CCATCAAGAA ACAGCTGGAG GAACCAGGGT TAGAGACAGG
   TAGGTGGAAC TGGTCTGCTT TGTATGCTCC A
      AGGACCCAGG GTCATGTACA GGTAGGCAGA GCTGGTCTG
      AGGACCCAGG GTCTTGTACA GGTAGGCAGA GCTGGTCTG
      AGGACCCAGG GTCTTGTACA GGTAGGCAGA GCTGGTCTG
      AGGACCCAGG GTCTTGTACA GGTAGGCAGA GCTGGTCTG
      AGGACCCAGG GTCTTGTACA GGTAGGCAGA GCTGGTCTG
      AGGACCCAGG GTCTTGTACA GGT-GGCAGA GCTGGTCTG
      AGGACCCAGG GTCTTGT-CA GGTAGGCAGA GCTGGTCTG
      AGGACCCAGG GTCGTGTACA GGT
                TGGTA GAGTTCTCCC ATCAGGTGTG GTCTGAAGAC
  CAGGGTCAAG CCACACAGGT AGGTAGGGTT GGCCCCTGAG GTATGGTTGG
  AGGAGCCAGG GTCAGAGACA GGTAGGCAGA GCTGGTCTGC TAGGAATGGT
  CTGAAGAGCC AAGGGTCATG TACAGGTAGG TGGAGCTGCT CTACCAGGTA
  TGCTCTCACC ACCCAGGATC ATGTACAGGT AGGCAGAGCT TGTTTTCTAA
 TTATGGTCTG ATCTCCCAGG GTCATGTACA GGTAGGTAAG AGTGGGCCTA
  TCAGGGATGG TCTGAAGAGC CAGGGTCAAG CCACGCA 3′

Consensus
      AGGACCCAGG GTCTTGTACA GGTAGGCAGA GCTGGTCTG
```

Figure 1. DNA sequence from the glucosephosphate isomerase intron containing the near perfect tandem repeat (in bold type).

The 39-bp tandem repeat does not show homology to the 15/18 bp repeat described by Coppieters *et al.* (1990) nor the 'core' sequences of other tandem repeats (Nakumura *et al.* 1987; Vassart *et al.* 1987; Jeffreys *et al.* 1985b).

The clone detects a polymorphism of at least six alleles in Southern blots (Fig. 2). The frequencies of the different alleles in 25 unrelated pigs were: allele 1 (3·8 kb) 0·16, allele 2 (3·0 kb) 0·04, allele 3 (2·8 kb) 0·20, allele 4 (2·7 kb) 0·12, allele 5 (2·6 kb) 0·28 and allele 6 (2·4 kb) 0·16. Mendelian inheritance has been demonstrated in several families (results not shown).

A high background was obtained at low stringency washing but no indication of multiple bands was detected.

In a gene mapping context, a useful marker for the centromeric region of chromosome 6 has been isolated. The presence of this tandem repeat has previously

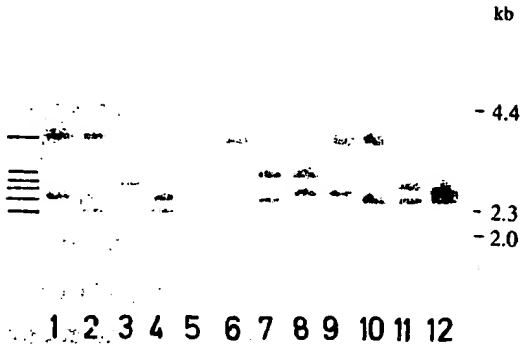

Figure 2. Autoradiogram of Southern blots of *Bam*HI digested pig DNA hybridized with the tandem repeat probe pPD05. Lanes 1 to 12 are DNA from unrelated individuals. The different alleles are indicated at the right hand side.

been described (Davies *et al.* 1988) and other RFLPs for the GPI locus reported (Brenig *et al.* 1990a,b; Archibald & Bowden 1991). However, the probe pPD05 detects bands in the 2–4 kb size range, compared to 6–9 kb previously published. This increases the degree of resolution and allows the detection of at least six alleles, increasing the information content of this RFLP.

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References

Ansorge W., Sproat B.S., Stegeman J. & Schwager C. (1986) A nonradioactive automated method for DNA sequence determination. *Journal of Biochemical and Biophysical Methods* **13**, 315–23.

Ansorage W., Sproat B.S., Stegeman J., Schwager C. & Zenke M. (1987) Automated DNA sequencing: ultrasensitive detection of fluorescent bands during electrophoresis. *Nucleic Acids Research* **15**, 4593–602.

Archibald A.L. & Bowden L.M. (1991) Molecular genetic markers for the halothane-induced malignant hyperthermia in pigs. *Animal Genetics* **22**, (Suppl. 1), 97.

Bell G.I., Selby M.J. & Rutter W.J. (1982) The highly polymorphic region near the human insulin gene is composed of simple tandem repeating sequences. *Nature* **295**, 31–35.

Birnboim H.C. & Doly J. (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* **7**, 1513–1523.

Brenig B., Jurs S. & Brem G. (1990a) The porcine PHIcDNA linked to the halothane gene detects a HindII and XbaI RFLP in n rmal and malignant hyperthermia susceptible pigs. *Nucleic Acids Research* **18**, 388–388.

Brenig B., Jurs S. & Brem G. (1990b) The porcine PHIcDNA linked to the halothane gene detects a NotI RFLP in normal and malignant hyperthermia susceptible pigs. *Nucleic Acids Research* **18**, 388.

Coppieters W., Van de Weghe A., Depicker A., Bouquet Y. & Van Zeveren A. (1990) A hypervariable pig DNA fragment. *Animal Genetics* **21**, 29–38.

Davies W., Harbitz I. & Hauge J.G. (1987) A partial cDNA clone for porcine glucosephosphate isomerase: isolation, characterization and use in detection of restriction fragment length polymorphism. *Animal Genetics* **18**, 233–240.

Davies W., Harbitz I., Fries R., Stranzinger G. & Hauge J.G. (1988) Porcine malignant hyperthermia carrier detection and chromosomal assignment using a linked probe. *Animal Genetics* **19**, 203–12.

Feinberg A.P. & Vogelstein B. (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry* **132**, 6–13.

Jeffreys A.J., Wilson V. & Thein S.L. (1985a) Individual specific 'fingerprints' of human DNA. *Nature* **316**, 76–78.

Jeffreys A.J., Wilson V. & Thein S.L. (1985b) Hypervariable 'minisatellite' regions in human DNA. *Nature* **314**, 67–73.

Kirstensen T., Voss H., Schwage C., Stegeman J., Sproat B. & Ansorge W. (1988) T7 polymerase in automated DNA dideoxy sequencing. *Nucleic Acids Research* **16**, 3487–96.

Miller S.A., Dykes D.D. & Polesky H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**, 1215.

Nakamura Y., Leppert M., O'Connell P., Wolff R., Holm T., Culver M., Martin C., Fujimoto E., Hoff M., Kumlin E. & White R. (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. *Sciences* **235**, 1616–22.

Vassart G., Georges M., Monsieur R., Brocas H., Lequarre A.S. & Christophe D. (1986) A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. *Science* **235**, 683–4.